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Investigation of local ACE activity and structural alterations during development of L-NAME-induced hypertension

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Abstract

Tonic basal release of nitric oxide (NO) by vascular endothelial cells controls blood pressure (BP) in the basal state. In the present study, we showed how serum and local angiotensin converting enzyme (ACE) alters during development of hypertension in chronic nitric oxide synthase blockade, a non-renin-dependent model of hypertension. Four experiments were performed in which animals were given N^{ω}-nitro-L-arginine methyl ester (L-NAME) (50 mg kg^{-1}) for 2, 4, 8 and 12 weeks. The control group rats received tap water. The ACE activity in serum, aorta, heart, kidney and lung was analyzed by high performance liquid chromatography (HPLC) and the structural change in aorta was investigated by measurement of cross-sectional area (CSA). Significant elevation of systolic blood pressure developed in chronically NO-blocked rats compared to controls. These results indicated that ACE activity in aortae and heart was gradually increased during development of hypertension and was more pronounced at higher blood pressure. Furthermore, there was a positive correlation between aortic cross-sectional area and elevation of blood pressure. These observations highlight the importance of the local ACE particularly in organs regulating hypertension (aorta and heart) during development of L-NAME-induced hypertension.

Keywords: Nitric Oxide; Tissue ACE activity; Hypertension

1. Introduction

Despite the fact that essential hypertension is one of the most prevalent diseases and an important risk factor for cardiovascular morbidity and mortality, the underlying pathophysiological abnormalities leading to the development of the elevated arterial pressure in this disorders remain elusive [22].

In normal blood vessels, endothelium-derived nitric oxide (NO) is continuously released both luminally and abluminally, protecting against platelet aggregation and adhesion [12,14] as well as influencing vascular smooth muscle tone [7,20]. NO is enzymatically synthesized from L-arginine, a process that can be antagonized by substituted L-arginine compounds such as *N*-nitro-L-arginine methyl ester (L-NAME), which compete for the NO synthase [18]. Several studies have demonstrated that blockade of NO synthesis leads to a marked rises in systemic blood pressure (BP) [21], suggesting that tonic release of NO controls BP in the resting state. Rats chronically treated with L-NAME provide a new model of hypertensive disease, with decreased NO synthase activity as well as chronic decrease in the arterial wall cyclic GMP content [3]. The NO synthesis blocker, L-NAME, is water soluble and orally active and when given in the drinking water to animals produces a prolonged increase in blood pressure over many hours [6].

The renin angiotensin aldosterone system (RAAS) plays a major physiologic role in blood pressure control and sodium volume homeostasis [23]. This system has been suggested to be of importance in pathologic conditions such as high blood pressure and renal complications [17]. Angiotensin converting enzyme (ACE) is a zinc metallopeptidase which plays a major role in regulation of vascular tone by converting the inactive peptide, angiotensin I (Ang I) into vasoconstrictor

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and trophic angiotensin II (Ang II) [15]. The octapeptide Ang II is the active component of the renin–angiotensin system (RAS) and has a major role in the regulation of cardiovascular, renal and endocrine functions [8]. ACE exists both as a membrane-bound enzyme in various organs such as heart, blood vessels, kidney and in a freely soluble form in plasma [11,28]. There are some reports suggesting that RAS is altered in this model of chronic administration of L-NAME [1,27]. It has been shown that blockade of the RAS with a converting enzyme inhibitor [13], or with an angiotensin II (Ang II) receptor antagonist [19] prevents the elevation of BP in L-NAME-treated animals. It has also been reported that gene expression of angiotensin II receptor increased in L-NAME-received animals [5]. Furthermore, several reports have shown that NO antagonizes the biological function of Ang II, such as migration of vascular smooth muscle cells [11]. However, less is known about alterations of serum and tissue ACE activity during development of L-NAME-induced hypertension. In this study, the role of ACE activity in serum and various organs including kidney, heart, aorta and lung were investigated during development of hypertension and particularly the correlation between this enzyme activity and structural changes in the aorta of L-NAME-treated animals were also been examined.

2. Material and methods

2.1. Animals

Five groups of normotensive age-matched Sprague–Dawley rats weighing 200–250 g were used in this study ($n = 10$). All of animals had free access to water and standard rat chow. The first group of rats received L-NAME (50 mg/100 ml, Sigma Chemical, St. Louis, MO, USA) in the drinking water for 2 weeks (W1). Their daily intake of L-NAME was $\sim$50 mg kg^{-1}. The other groups received the same amount of L-NAME for 4, 8 and 12 weeks correspond to W2, W3 and W4 groups, respectively. The respective control groups received tap water for the same periods.

2.2. Measurement of blood pressure

To assess the development of hypertension, indirect systolic tail-cuff blood pressures were routinely obtained with a Narco Bio-systems Electro-Sphygmomanometer (Huston, TX, USA). The mean of three measurements was obtained from each rat once a week until the end of the experiment.

2.3. Tissue preparation and measurement of ACE activity

Blood samples were collected for measuring serum ACE activity and rats were then sacrificed by decapitation under ether general anesthesia. Kidney, heart, lung and a 1-cm segment of thoracic aorta were rapidly removed, cleaned of fatty and connective tissues, blotted dry and then weighted. ACE activity was measured as described by Horiuchi et al. [9]. Tissues were homogenized at 4 °C in cold Trizma–HCl buffer (pH 7.8) containing 30 mM KCl, 5 mM magnesium acetate, 0.25 M sucrose and 1% of Triton X-100 (Sigma). The homogenate was centrifuged at 5000 × g for 15 min at 4 °C and supernatant was used for assay. Serums and supernatants of centrifuged samples were kept frozen at −80 °C until assayed.

ACE activity was determined by high performance liquid chromatography (HPLC). Briefly 40 μl of borate buffer containing 3.5 mM P-benzoyl-L-glycyl-L-leucine (Hip-His-Leu, Sigma) as substrate was added to the 10 μl of sample and incubate at 37 °C for 30 min with constant shaking. The reaction was stopped by addition of 150 μl of methaphosphoric acid and then was centrifuged at 2000 × g for 5 min and 20 μl of supernatant was injected into the column and the amount of hippuric acid liberated from the substrate was analyzed by HPLC. One unit of activity is defined as the amount of enzyme catalyzing the release of 1 μM of hippuric acid from Hip-His-Leu per minute at 37 °C under standard assay conditions.

2.4. Histological evaluation

The thoracic aorta was dissected out, cleaned and fixed in 10% formaline. The aortic tissue then were embedded in paraffin and sectioned into 8 μm thick sections and stained with hematoxylin–eosin (H&E) for light microscopic study. The cross-sectional area (CSA) of aorta was evaluated from photographs of whole aortic ring sections taken at 40× magnification and scanned, digitized and analyzed by computer, using the Adobe Photoshop Imaging program (Adobe System Incorporation) [25].

2.5. Statistical analysis

For SBP determination, the average of three measurements was taken at the time of recording. The data presented was the mean ± S.E.M. Average of blood pressure in each group compared to respective control group using an unpaired Student t-test. Also comparison of heart weight (HW), and aortic weight (AW) of L-NAME-induced hypertensive rats and their age-matched controls were made by unpaired Student t-test.

Multiple comparisons of tissues and serum ACE activity with their control values were made using one-way analysis of variance (ANOVA). The relationship between variables was assessed by a simple linear regression analysis. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Blood pressure and tissue weight

Time-related changes in systolic arterial pressure are shown in Fig. 1. In L-NAME-treated animals, the SBP

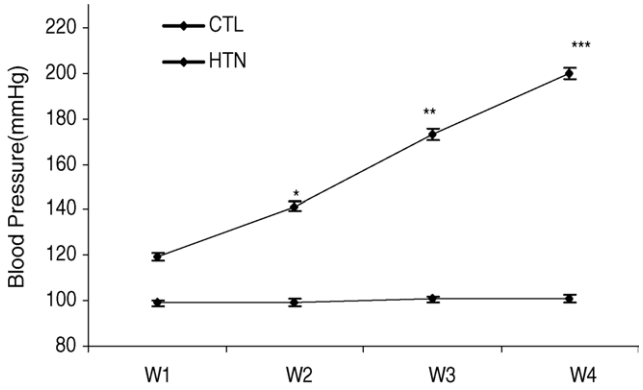

Fig. 1. Time-related changes in systolic arterial pressure in control (CTL) and L-NAME-received animals for 2, 4, 8 and 12 weeks correspond to W1, W2, W3 and W4 groups, respectively. Values are expressed as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the C group.

increased progressively, but no significant elevation in SBP was observed at 2 weeks after L-NAME administration compared to control. The SBP significantly enhanced in W2 and W3 groups ($P < 0.05$, $P < 0.01$, respectively) and reached to highest level during 12 weeks after L-NAME treatment ($P < 0.001$).

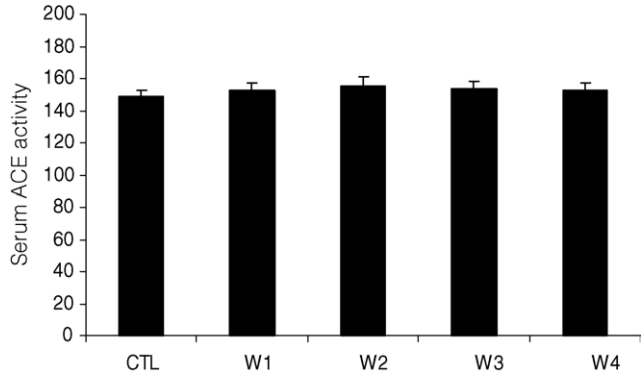

Fig. 2. Angiotensin-converting enzyme (ACE) activity (μmol/min g) of serum in control (CTL) and L-NAME-received animals for 2, 4, 8 and 12 weeks correspond to W1, W2, W3 and W4 groups. Values are expressed as mean ± S.E.M. ($n = 10$).

3.2. Serum and tissue ACE activity

ACE activity determined in serum from hypertensive rats taken at 2, 4, 8 and 12 weeks after L-NAME treatment was similar to that of normotensive control rats (Fig. 2).

The local ACE activity in different tissues during development of L-NAME-induced hypertension was measured

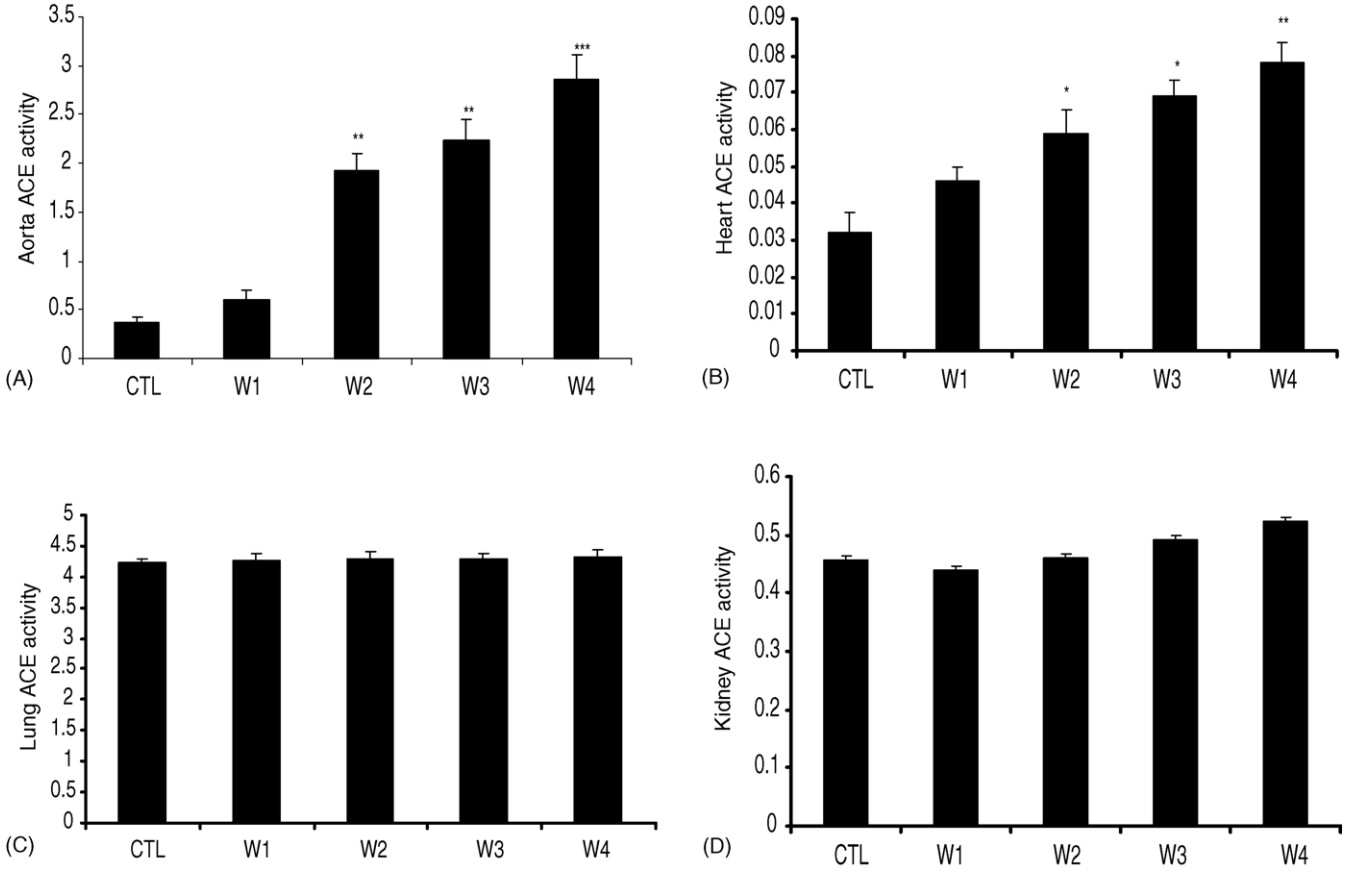

Fig. 3. Angiotensin-converting enzyme (ACE) activity (μmol/min g) of tissues including aorta (A), heart (B), lung (C) and kidney (D) in control (CTL) and L-NAME-received animals for 2, 4, 8 and 12 weeks correspond to W1, W2, W3 and W4 groups. Values are expressed as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared to respective control groups ($n = 10$).

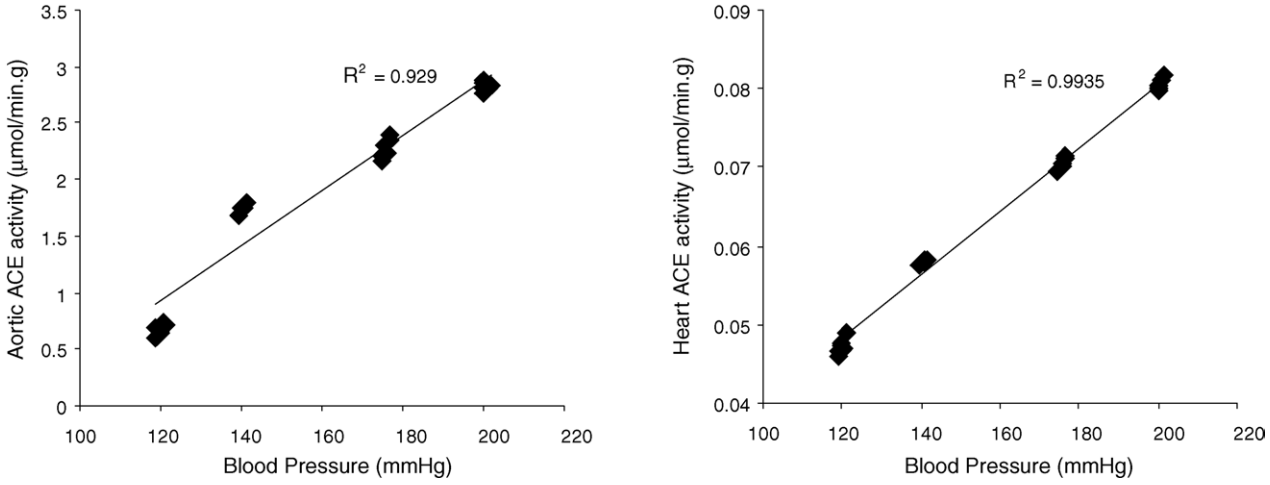

Fig. 4. Correlation between blood pressure and heart (A) and aortae (B) angiotensin-converting enzyme (ACE) activity in L-NAME-received animals for 2, 4, 8 and 12 weeks correspond to W1, W2, W3 and W4 groups, respectively ($n = 10$). Aortic ACE activity vs. blood pressure, $P < 0.001$ and heart ACE activity vs. blood pressure, $P < 0.001$.

(Fig. 3), in which ACE activity in aorta and heart homogenates but not in lung and kidney markedly increased in parallel to elevation of blood pressure. Also there was a positive correlation between elevation of blood pressure and alteration of ACE activity in the aortae and hearts of L-NAME-induced hypertensive rats (Fig. 4). The correlation coefficients between ACE activity and augmentation of blood pressure were 0.92 and 0.99 for aorta and heart, respectively.

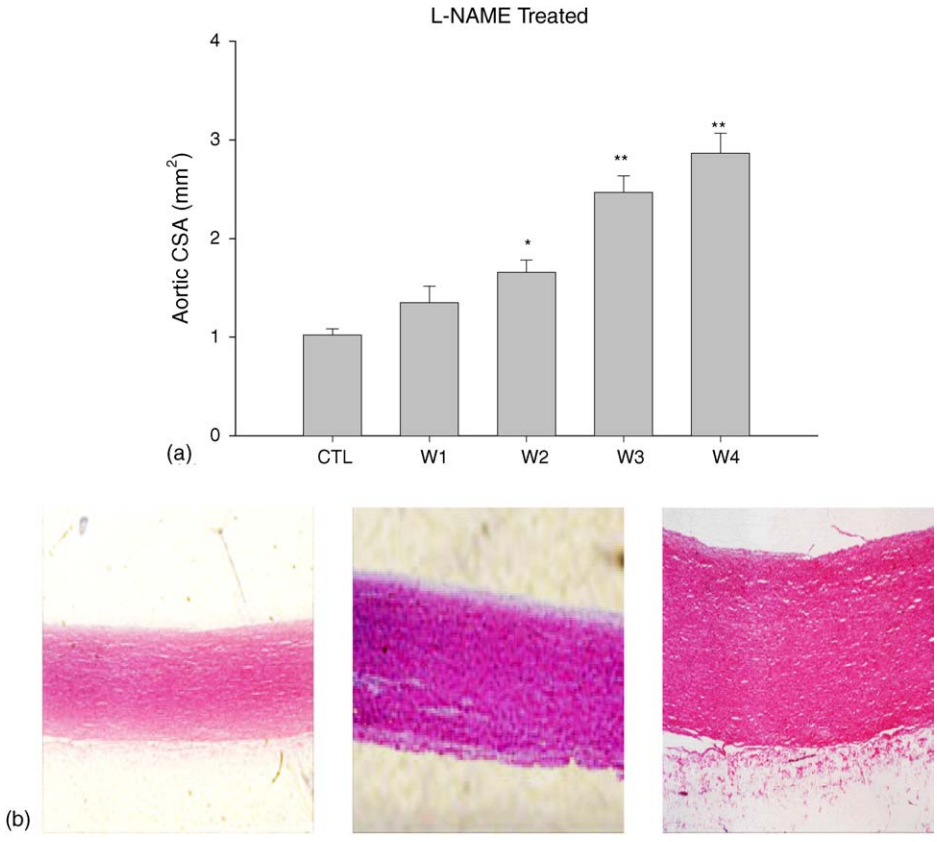

Fig. 5. (a) Bar graphs show the CSA of thoracic sections of 10 rats per groups. Values are expressed as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group. (b) Micrographs of cross-sectional area (CSA) of thoracic aorta (original magnification 40×) from (A) control group, (B) L-NAME-treated rats at 8 weeks (W3), and (C) L-NAME-treated rats at 12 weeks (W4).

Table 1
Comparison of body weight (BW), heart weight (HW), and aortic weight (AW) of L-NAME-induced hypertensive rats during 2, 4, 8 and 12 weeks and age-matched controls (CTL)

	Time (weeks)							
	2		4		8		12	
	CTL	L-NAME	CTL	L-NAME	CTL	L-NAME	CTL	L-NAME
BW (g)	240 ± 2.9	231 ± 4.6	268 ± 3.3	273 ± 5.2	319 ± 7.9	332 ± 9.1	347 ± 9.9	360 ± 8.7
AW (mg)	44 ± 2.3	59 ± 1.9	47 ± 3.0	72 ± 2.9*	51 ± 1.1	93 ± 3.4*	54 ± 2.4	1.13 ± 3.6**
HW (mg)	622 ± 9.0	693 ± 11	629 ± 10	946 ± 15*	638 ± 18	1263 ± 19**	643 ± 16	1396 ± 22**

Data are presented as mean ± S.E.M.
* $P < 0.05$.
** $P < 0.01$ vs. control group.

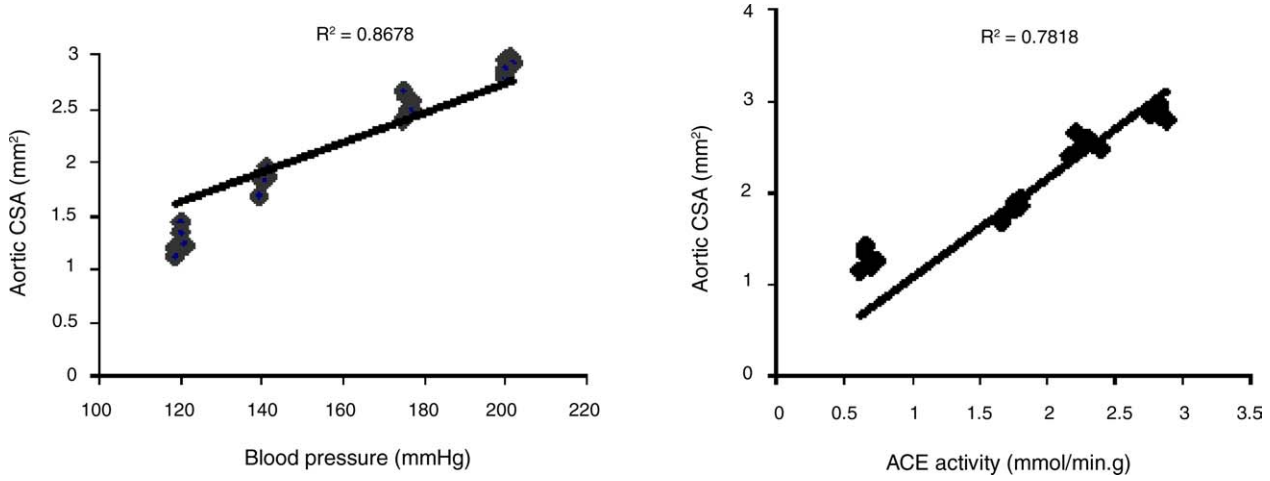

Fig. 6. Correlation between aortic cross-sectional area (CSA) and blood pressure (A) and aortic ACE activity (B) angiotensin-converting enzyme (ACE) activity in L-NAME-received animals for 2, 4, 8 and 12 weeks correspond to W1, W2, W3 and W4 groups, respectively ($n = 10$). Aortic CSA vs. blood pressure, $P < 0.01$ and aortic CSA vs. ACE activity, $P < 0.01$.

3.3. Tissue weight

The heart and aortic weight significantly increased during development of chronic NO-blockade while body weight did not show marked alterations in hypertensive animals (Table 1).

The aortic CSA (mm^2) was increased in L-NAME-induced hypertensive animals (Fig. 5a). It was significantly greater in W2 ($P < 0.05$), W3 ($P < 0.01$) and W4 ($P < 0.001$) L-NAME-induced rats compared to controls and was correlated with augmentation of aortic ACE activity and amount of blood pressure during development of hypertension (Fig. 6).

4. Discussion

This study investigates the alterations of ACE activity in serum as well as other tissues including heart, aorta, kidney and lung during 2, 4, 8 and 12 weeks after L-NAME-induced hypertension in rats. The structural changes of aorta were also evaluated during development of hypertension. In present study, the major finding was the augmentation of ACE activity in cardiac and aortic tissues during development of L-NAME-induced non-renin-dependent hypertension, while serum, kidney and lung ACE activity was unchanged.

It is now well recognized that the renin–angiotensin system is not only an endocrine system, but also most of its components are generated or activated in several tissues. It has previously been demonstrated by our group that local ACE activity was elevated in various organs of two-kidney, one-clip (2K1C), a renin-dependent and one-kidney, one-clip (1K1C), a non-renin-dependent model of hypertension [24,29]. In addition to that, the major role of vascular ACE activity has also been previously reported in spontaneously hypertensive rats [17].

It has widely been shown that long-term administration of L-NAME in rats could decrease NO synthesis and cause arterial hypertension [21]. The present study demonstrated that the structural changes in aorta started from fourth week of L-NAME administration, which was correlated with elevation of aortic ACE activity (Fig. 3A). Thus, long-term administration of L-NAME cause greater wall-to-lumen ratio in aorta and possibly activation of aortic ACE preceded the development of structural changes induced by higher angiotensin II. Such evidences suggest that inhibition of activated ACE may

explain the benefits of ACEI in lowering blood pressure of NO-blocked animals [13,19].

These findings are in agreement with previous reports which demonstrated development of vascular structural changes and cardiac hypertrophy with increased ACE activity [2]. In the present study, tissue ACE activities were increased only in the heart and aorta but not in other organs examined. A significant correlation was also been observed between aortic structural changes and ACE activity (Fig. 6).

It has been demonstrated that nitric oxide decrease vascular response to Ang II through cGMP production [26]; conversely acute blockade of NO synthase enhances the response to Ang II [10]. Hence, it appears that both a reduced production of nitric oxide and an enhanced release of vasoconstriction factors such as Ang II contribute to the impaired endothelium-dependent relaxations in L-NAME-induced hypertensive rats.

Previous investigations considered two pathophysiological stages for L-NAME-induced hypertension. First, an early stage in which SBP is high but there are no changes in the renin activity and there is no cardiovascular hypertrophy [16,2]. In the later stage, there is a gradual elevation of renin activity associated with structural damage in vasculature of the kidney and central nervous system leading the death [16]. Our results in agreement with previous findings have shown that cardiac and aortic ACE activity (but no other tissues) gradually increased during chronic phase of NO blockade. It is suggested that the activation of cardiac and possibly aortic ACE may contribute in development of high blood pressure and vascular structural changes in this model. In another world, our data propose a possible link between NO synthesis and tissue ACE activity in development of hypertension and underlying structural changes. However, the mechanisms by which ACE is activated after long-term administration of L-NAME were not explored in this study and need to be explored by further investigation.

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